



# Phycobiliproteins: Structural aspects, functional characteristics, and biotechnological perspectives



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## ABSTRACT

Phycobiliproteins (PBPs) are fluorescent proteins of various colors, including fuchsia, purple-blue and cyan, that allow the capture of light energy in auxiliary photosynthetic complexes called phycobilisomes (PBS). PBPs have several highly preserved structural and physicochemical characteristics. In the PBS context, PBPs function is capture luminous energy in the 450–650 nm range and delivers it to photosystems allowing photosynthesis take place. Besides the energy harvesting function, PBPs also have shown to have multiple biological activities, including antioxidant, antibacterial and antitumours, making them an interesting focus for different biotechnological applications in areas like biomedicine, bioenergy and scientific research. Nowadays, the main sources of PBPs are cyanobacteria and micro and macro algae from the phylum Rhodophyta. Due to the diverse biological activities of PBPs, they have attracted the attention of different industries, such as food, biomedical and cosmetics. This is why a large number of patents related to the production, extraction, purification of PBPs and their application as cosmetics, biopharmaceuticals or diagnostic applications have been generated, looking less ecological impact in the natural prairies of macroalgae and less culture time or higher productivity in cyanobacteria to satisfy the markets and applications that require high amounts of these molecules. In this review, we summarize the main structural characteristics of PBPs, their biosyntheses and biotechnological applications. We also address current trends and future perspectives of the PBPs market.

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## 1. Introduction

The largest source of energy on our planet comes from the sun in the form of light, in form of indivisible particles called photons. Another way to define light is electromagnetic energy, which can be detected by the human eye at ranges from 400 nm to 700 nm, approximately. This range is used by several organisms to perform photosynthesis. [1]

Photosynthesis is a biological oxide-reduction process, in which chemical energy is obtained from inorganic compounds as a substrate and the electromagnetic energy of light, captured using specialized pigments. The energy captured by these pigments is transformed and stored in different compounds, mainly carbohydrates, as chemical energy [2].

Chlorophyll (Chl), the main photosynthetic pigment, is associated with multiprotein complexes known as reaction centers of photosystems [3,4]. Chl is made up of a porphyrin ring formed

by four pyrrolic rings. It also has a magnesium ion associated with the pyrrolic rings through coordinate covalent bonds and a hydrocarbon chain known as phytol (Fig. 1A) [5,6]. All photosynthetic organisms contains Chlorophyll *a*, since they all derived from a common ancestor [7]. To date, six different types of chlorophylls have been described (a, b, c1, c2, d, and f), varying in the radical groups attached to the carbon atoms of their porphyrin ring resulting in different spectrums of absorption between the different chlorophylls. All different Chls can efficiently absorb light energy between 400 and 500 nm and between 650 and 720 nm. Despite the great variety of Chls, they all have poor absorption of wavelength light energy in the range of 500 to 650 nm, a gap zone commonly known as the “Green Gap” (Fig. 1B) [4,8–10].

About 45% of photosynthesis on Earth is carried out in aquatic environments [7], mainly by microalgae. However, in these environments, the irradiance and spectral distribution of light are not uniform due to a series of physical phenomena such as reflection,

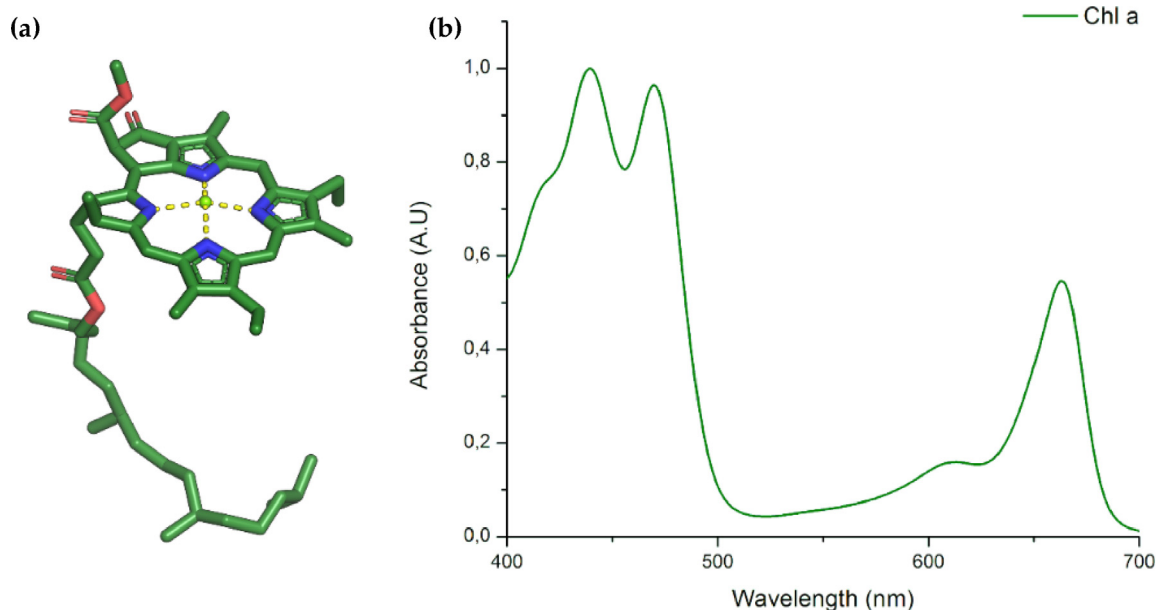


Fig. 1. (a) Molecular structure of chlorophyll *a*. (b) Absorption spectrum of Chlorophyll *a* from *Agarophyton chilense*. (Chlorophyll *a* molecule created with pymol from PDB:5B66. Absorption spectrum of Chl *a* made by the authors, unpublished data).

absorption, and scattering [1]. Organisms such as cyanobacteria and red algae have auxiliary photosynthetic complexes that allow to colonize environments where solar energy is not optimal to carry out photosynthesis. These complexes make it possible to capture green and yellow light (500 nm–600 nm) and later transfer it to Chl molecules, allowing these organisms to carry out photosynthesis and populate ecological niches that could not be colonized by organisms that only have chlorophyll as their main photosynthetic pigment. Among these light collecting accessory systems are phycobilisomes.

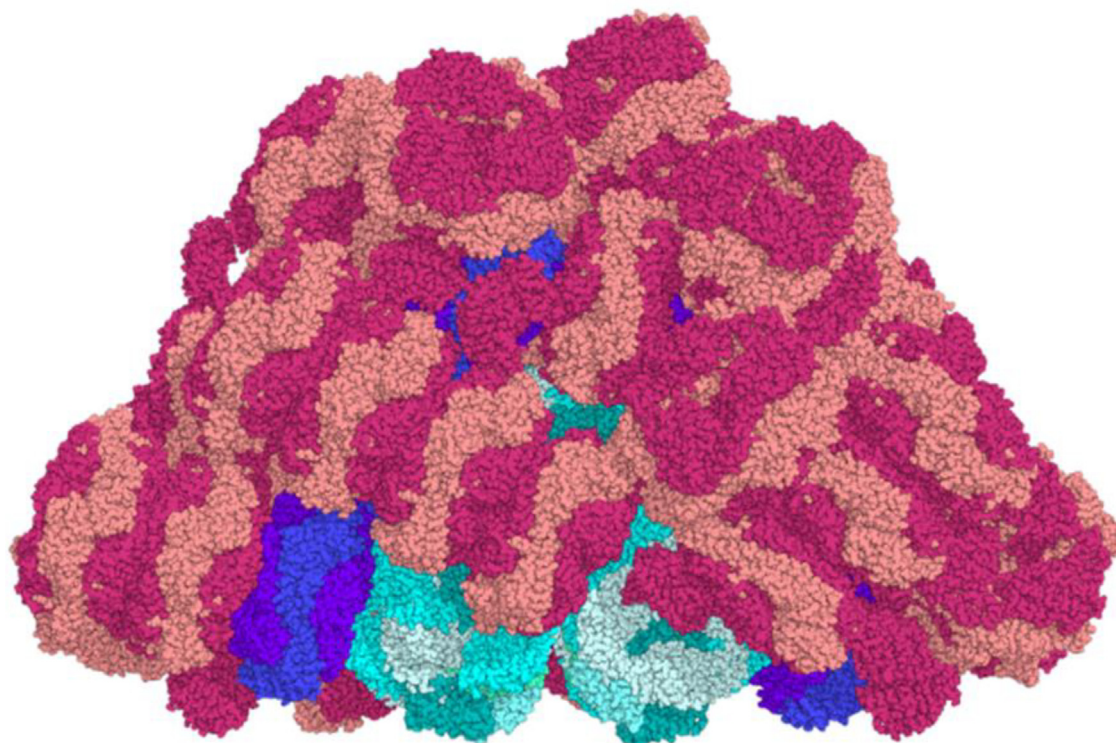
The phycobilisome (PBS) is a multiprotein complex, which efficiently captures light energy in the wavelength range of 450–650 nm, transferring it in a unidirectional way within its structure and delivering it to the Chl molecules present in photosystems [11]. The major component of phycobilisomes are phycobiliproteins (PBPs) (85%) and the remaining 15% corresponds to linker proteins [12]. PBPs are responsible for capturing and transferring light energy and linkers allow the correct assembly of the PBS and modulate energy transfer [13–18].

Electron microscopy experiments have shown that PBS possess 2 clearly defined substructures: the rods and the core [19–21]. The rods are composed of two or three PBPs, namely phycoerythrin (PE) and phycoerythrocyanin (PEC), these being the most distal PBPs from the core. The rods are also composed of phycocyanin (PC), which is the phycobiliprotein closest to the core. However, PE and PEC are not always part of the rods of the phycobilisomes. For example, there are some species of cyanobacteria, such as *Spirulina platensis*, whose rods only have PC and protein linkers that remain associated with the core of the phycobilisome [15]. The core is formed by allophycocyanin (APC), together with linker proteins. This core transfers the energy captured by the rods to the chlorophyll present in the thylakoid membrane [22].

In 2020 and 2021, the structure of the phycobilisomes of *Porphyridium purpureum* [17], *Anabaena* sp. PCC 7120 and *Synechococcus* sp. PCC 7002 [18] were reported. These structures provided new information on energy transfer within this complex. The PBS of *Porphyridium purpureum* is composed by more than 860 protein molecules (Fig. 2) and more than 2000 chromophores, forming a large complex with dimensions of approximately 680 Å wide, 350 Å height, and 450 Å deep. Its estimated mass has been reported to be close to 16.8 MDa [23,24].

PBPs are fluorescent hydrophilic proteins, characterized by having several chromophores called phycobilins attached to their polypeptide structure. Phycobilins consist of open chain tetrapyrroles derived from the heme group [25]. The phycobilins can have 64 different isomers which differ in configurations (Z/E) and conformations [sys (s)/anti (a)] [26], but the most common isomers in PBPs are ZZZ-ssa and ZZZ-asa isomers [17]. Phycobilins are covalently bonded to specific and highly preserved cysteine residues, which give PBPs their spectroscopic characteristics. There are four phycobilins, each showing different colors: Phycocyanobilin (PCB) is blue; phycoviolobilin (PVB) is violet, phycoerythroblin (PEB) is red and phycourobilin (PUB) is yellow [27]. Each phycobilin possess a system of conjugated double bonds that give it a spectroscopic characteristic. This characteristic depends on the number of conjugated double bonds that each phycobilin possesses, the greater the number of conjugated double bonds, the greater the shift in the absorption maximum towards red. The PCB molecule has nine conjugated double bonds, PVB has eight, PEB has six, and finally, PUB has five conjugated double bonds [28]. The phycobilins present in the PBPs are responsible for the absorption of light energy in the PBS, in the range between 500 and 650 nm. (Fig. 3).

The precise relationship in the absorption and emission spectrum of PBPs makes this light-collecting complex one of the most efficient in nature [23,32]. Recent studies related to PBS structure



**Fig. 2.** Structure of *Porphyridium purpureum* phycobilisome. In pink and light pink PE are shown. In Purple and light purple PC are shown. In turquoise and light turquoise APC are shown. (Phycobilisome image created with pymol from PDB: 6xwk). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

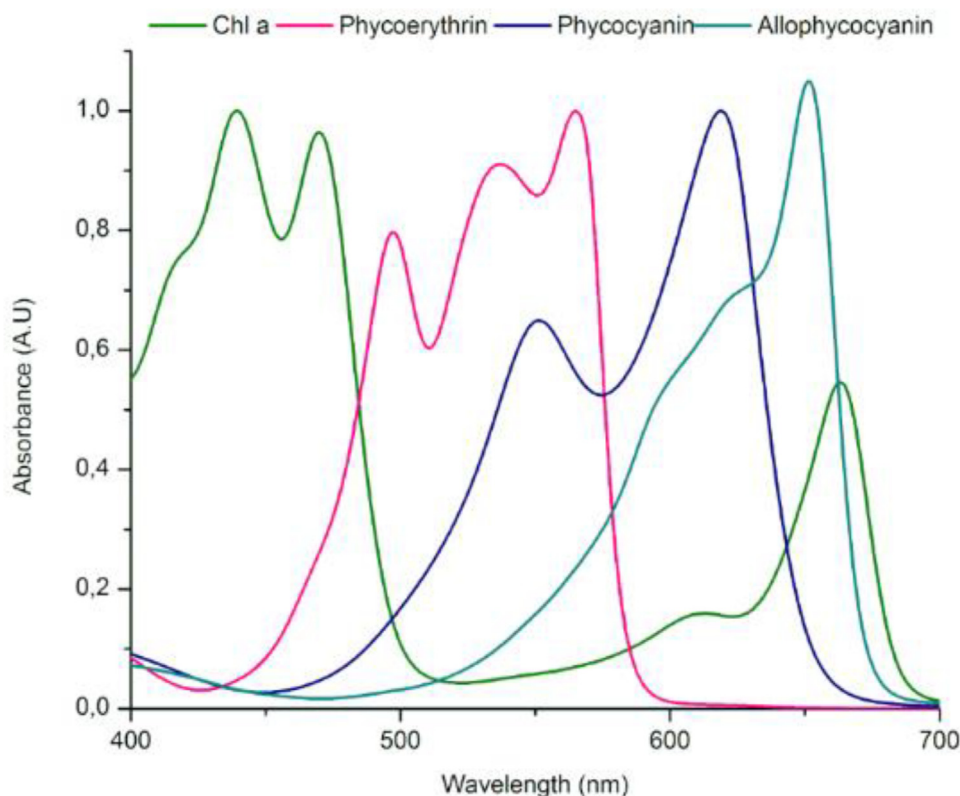


Fig. 3. Absorption spectrum of PBPs from rhodophytes. PE spectrum ref [29]. PC spectrum ref [30]. APC spectrum ref [31]

have helped to explain the high efficiency of energy transfer in this complex [17–18], these studies demonstrate that it is not only necessary to know the type and number of chromophores present in the PBS, but also the structural organization or the architecture of its components, as they play an important role. Another important factor in the energy transfer in the PBS is the geometry and spectroscopic properties of chromophores, together with the protein environment.

All PBPs share a series of very similar structural and functional characteristics. Knowing the structure features of PBPs, as the main component of PBS, is of high relevance in order to understand their biological activities and potential biotechnological uses. Several studies have reported numerous bioactive properties of PBPs, including antioxidant, anticancer, antimicrobial and anti-inflammatory activity. All these properties have contributed to a growing interest in PBPs in the pharmaceutical, food and cosmetic industries. In this context, the aim of this article is to provide an overview of the structural characteristics of PBPs, their biosynthesis and their spectroscopic properties and summarize PBPs biotechnological applications in the form of patents related to extraction, production, purification. Finally, future perspectives and food industry applications are briefly emphasized.

## 2. Structural characteristics of PBPs

To date, there are sixty-one phycobiliprotein structures deposited in the Protein Data Bank (PDB). Of these, twelve structures correspond to PE; three are from phycoerythrocyanin; thirty-six structures are phycocyanin and ten are allophycocyanin. All these structures have been obtained by means of the x-ray diffraction technique and most of them belong to cyanobacteria and red algae. All these proteins structures shows that PBPs are composed of two

subunits, consisting of two polypeptide chains known as  $\alpha$  and  $\beta$ . These subunits show similar basic structures, with 6–8 helical zones separated by loops and a similar globulin-like fold [33]. Functionally, each subunit can have one to three phycobilin molecules attached to the polypeptide skeleton in highly preserved cysteine residues (50, 60, 81 and 150), but Cys 81 residue always shows a bonded phycobilin [34]. Fig. 4 shows a structural alignment of APC (PDB: 6YX7), PC (PDB: 6XWK), PEC (PDB: 2C7L) and PE (PDB: 1B8D) and with their respective secondary structure elements of each PBP. From this alignment, the cysteine residue which is always chromophorylated is highlighted in green. Along with the above, the alpha helices of each of the subunits of the different PBPs are shown as red cylinders. The number of the  $\alpha$ -helices of each structure was obtained from the information reported in the PDB by the authors of the respective crystallographic structures. The structural alignment was done with the PDBeFold server and the visualization of the alignment was done with Jalview. The selection of the structures used in this alignment was made based on the criterion of best resolution of the crystallographic structure.

The analysis of amino acid sequences and the structures of different phycobiliprotein subunits suggests that they come from a common ancestor and that the duplication of the ancestral gene gave rise to the  $\alpha$  and  $\beta$  subunits of the different phycobiliproteins [33]. Despite the low similarity of the subunit sequences, there are a series of residues that remained unchanged, as they play a critical role in the structure or function of the phycobiliprotein, such as the chromophore binding site and residues of the interface surface between the  $\alpha$  and  $\beta$  subunits [33].

From a physicochemical point of view, the subunits of the different PBPs have molecular weights that range between 16,000 and 18,000 Dalton and with an isoelectric point close to 6.  $\alpha$



**Table 1**

Physicochemical, sequence comparison and biological assembly information of PBPs present in the protein data bank (PDB). CB means Cyanobacteria, RA means Red algae. In bold are show the structure used for the sequence and RMSD analysis.

Phycobiliprotein	Specie	Subunit	Residues	Molecular Mass (Da)	Isoelectric Point	% Sequence Identity/amino acids similarity	RMSD (Å)	Biological assembly	PDB code
<b>Phyco erythrin</b>	<i>Gloeobacter Violaceus</i> (CB)	α	164	17658.04	6.73	23,7/52,5	2, 18	(αβ) <sub>6</sub>	2VJH
		β	177	18427.16	7.52				
	<i>Phormidium rubidum</i> A09DM (CB)	α	164	17643.87	5.84	25/44,5	2,06	(αβ) <sub>6</sub>	<b>5NB4</b> , 5NB3, 5AQD, 5FVB
		β	184	19336.16	6.57				
	<i>Agarophyton chilense</i> (RA)	α	164	17751.88	5.42	27/48,6	2, 13	(αβ) <sub>6</sub>	1EYX
		β	177	18604.18	5.15				
	<i>Griffithsia monilis</i> (RA)	α	164	17668.86	5.69	24,5/46,8	2, 13	(αβ) <sub>6</sub>	1B8D
		β	177	18481.94	5.10				
	<i>Palmaria palmata</i> (RA)	α	164	17638.79	5.40	24,6/47,6	2, 14	(αβ) <sub>6</sub>	5B13
		β	177	18408.00	5.42				
<i>Polysiphonia urceolata</i> (RA)	α	164	17836.07	5.70	25/50,5	2, 14	(αβ) <sub>6</sub>	<b>1LIA</b> , 1F99	
	β	177	18721.38	5.15					
<i>Porphyridium cruentum</i> (RA)	α	164	17805.05	5.42	27,2/51,1	2, 12	(αβ) <sub>3</sub>	3 V57, 3 V58	
	β	177	18554.18	5.43					
<b>Phyco Erythro cyanin</b>	<i>Mastigocladus laminosus</i> (CB)	α	162	17563.74	6.82	27,5/44,4	2,00	(αβ) <sub>3</sub>	<b>2C7L</b> , 2C7K, 2C7J
	β	172	18486.95	6.72					
<b>Phyco cyanin</b>	<i>Acaryochloris marina</i> (CB)	α	162	17375.43	5.28	26,9/52,6	2,01	(αβ) <sub>3</sub>	50OK
	β	172	18022.41	4.94					
<i>Cyanidium caldarium</i> (CB)	α	162	17505.66	5.81	27,6/54	1,91	(αβ) <sub>6</sub>	1PHN	
	β	172	18252.74	4.96					
<i>Gloeobacter Violaceus</i> (CB)	α	162	17662.90	7.74	23,7/53,2	2,03	(αβ) <sub>6</sub>	<b>2VML</b> , 2VJR	
	β	172	18459.99	5.46					
<i>Leptolyngbya sp</i> (CB)	α	162	17585.87	5.83	24,6/45	1,78	(αβ) <sub>6</sub>	<b>4L1E</b> , 2UUN	
	β	172	18066.54	4.80					
<i>Microchaete diplosiphon</i> (CB)	α	162	17228.34	6.56	24,6/52,6	1,66	(αβ) <sub>6</sub>	1CPC	
	β	172	17920.38	5.10					
<i>Phormidium rubidum</i> A09DM (CB)	α	162	17308.34	4.64	24,7/52,2	1,88	(αβ) <sub>6</sub>	<b>6XWK</b> , 2UUL	
	β	172	18124.49	5.35					
<i>Pseudanabaena sp</i> (CB)	α	162	17222.29	5.79	22,9/51,4	1,82	(αβ) <sub>6</sub>	5TOU	
	β	172	17842.27	5.40					
<i>Arthrospira platensis</i> (CB)	α	162	17601.87	5.83	25,7/45,5	1,82	(αβ) <sub>6</sub>	<b>1HA7</b> , 1GH0, 2UUM	
	β	172	18093.57	4.96					
<i>Synechococcus elongatus</i> (CB)	α	162	17442.70	5.36	27/52,3	1,77	(αβ) <sub>6</sub>	<b>3LOF</b> , 4ZIZ, 4Q70, 5MJP, 5MJM, 5O7M, 4Z8K, 5MJQ, 5UVK, 4H0M, 1JBO, 4F0T	
	β	172	18186.67	5.12					
<i>Synechocystis sp. PCC 6803</i> (CB)	α	162	17586.60	5.35	26,9/49,7	2,07	(αβ) <sub>3</sub>		
	β	172	18126.47	4.98					
<i>Thermo synechococcus vulcanus</i> (CB)	α	162	17456.72	5.36	26,4/52,3	1,74	(αβ) <sub>3</sub>	<b>3O18</b> , 3O2C, 1KTP, 4N6S, 4GY3, 11Y7, 1ON7, 4GXE, 2BV8	
	β	172	18186.67	5.12					
<i>Agarophyton chilense</i> (RA)	α	162	17576.64	5.15	25,4/53,7	1,90	(αβ) <sub>6</sub>		
	β	172	18162.25	4.65					
<i>Galdieria Sulphuraria</i> (RA)	α	162	17506.65	6.57	27,6/54	1,84	(αβ) <sub>6</sub>	3KVS	
	β	172	18224.69	4.96					
<i>Polysiphonia urceolata</i> (RA)	α	162	17569.78	5.81	27,5/56,7	1,85	(αβ) <sub>6</sub>	1F99	
	β	172	18000.54	4.92					
<b>Allophycocyanin</b>	<i>Arthrospira platensis</i> (CB)	α	161	17220.67	5.07	37/64,2	1,48	(αβ) <sub>3</sub>	1ALL
	β	161	17243.70	7.67					
<i>Phormidium rubidum</i> A09DM (CB)	α	161	17475.92	4.85	34,6/62,3	1,53	(αβ) <sub>3</sub>	4RMP	
	β	161	17408.82	5.15					
<i>Gloeobacter Violaceus</i> (CB)	α	161	17522.09	4.92	38,3/66	1,41	(αβ) <sub>3</sub>	2VJT	
	β	161	17211.69	6.24					
<i>Synechocystis sp. PCC 6803</i> (CB)	α <sup>B</sup>	161	17923.45	5.44	33,7/60,7	1,53	(αβ) <sub>3</sub>	4PO5	
	β	161	17215.64	5.43					
<i>Mastigocladus laminosus</i> (CB)	α	161	17252.63	4.90	38,3/65,4	1,77	(αβ) <sub>3</sub>	1B33	
	β	161	17373.84	5.45					
<i>Synechococcus elongatus</i> (CB)	α	161	17421.76	4.79	38/67,5	1,57	(αβ) <sub>3</sub>	4FOU	
	β	161	17392.77	5.45					
<i>Thermosynechococcus vulcanus</i> (CB)	α	161	17538.96	4.85	39,5/67,9	1,38	(αβ) <sub>3</sub>	<b>3DBJ</b> , 2V8A	
	β	161	17358.89	5.45					
<i>Agarophyton chilense</i> (RA)	α	161	17519.01	4.90	38,3/63,6	1,42	(αβ) <sub>3</sub>	5TJF	
	β	161	17471.82	5.10					
<i>Porphyra yezoensis</i> (RA)	α	161	17496.96	5.09	35,8/62,3	1,47	(αβ) <sub>6</sub>	1KN1	
	β	161	17443.89	5.45					

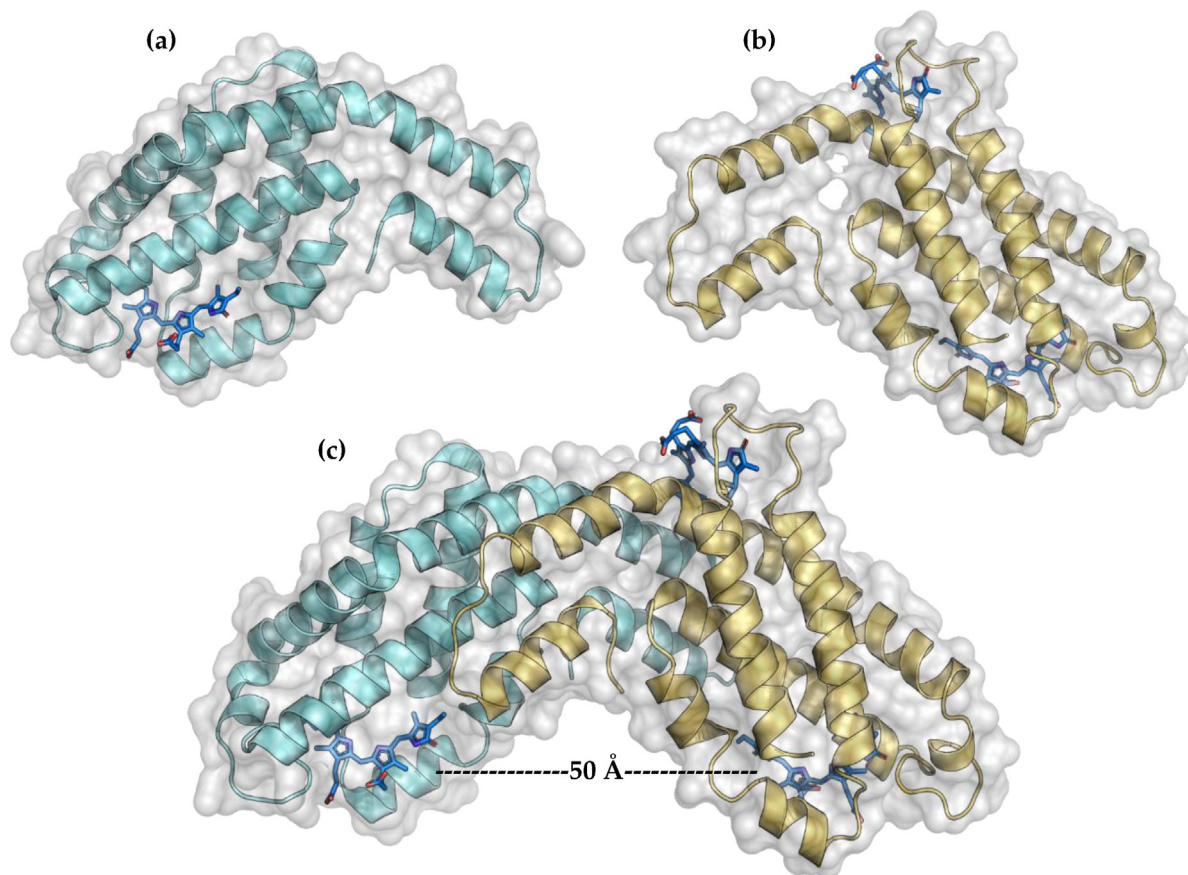


Fig. 5. Structural level of PBPs. (a) Subunit  $\alpha$ , (b) subunit  $\beta$ . (c) heterodimer  $\alpha\beta$ .

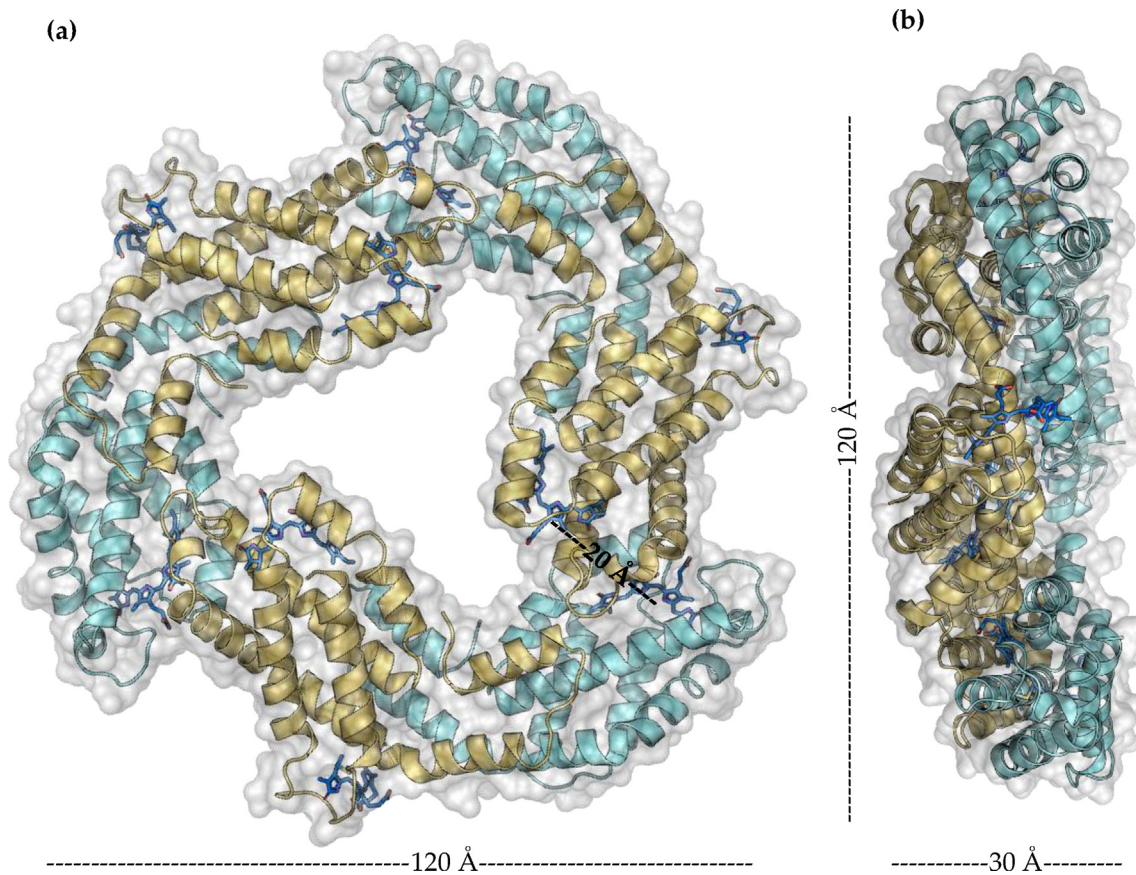


Fig. 6. Structural level of PBPs (a) trimer  $(\alpha\beta)_3$  front view. (b) trimer  $(\alpha\beta)_3$  side view.

possess a trimeric biological assembly. Table 1 shows data from biological assembly of PE, PEC, PC, and APC from structures deposited in the PDB.

### 2.1. Subunits and heterodimers ( $\alpha\beta$ )

The basic component of PBPs are subunits. Both  $\alpha$  and  $\beta$  subunits belong to all-alpha class, with a globin-like fold and are recognized as part of PF00502.

The analysis of different structures obtained by x-ray diffraction of PBPs heterodimers shows that the  $\alpha$  (Fig. 5 A) and  $\beta$  subunits (Fig. 5 B) stabilize each other forming an  $\alpha\beta$  heterodimer (Fig. 5 C). The approximate distance between the chromophores within

a heterodimer is approximately 50 Å, a large distance that prevents energy transfer within the heterodimer.

The area of interaction between the  $\alpha$  and  $\beta$  subunits is approximately 1400 Å<sup>2</sup>, formed by 35–40 residues and is stabilized by 12–15 hydrogen bonds. These interactions allow the formation of a very stable surface, which can only be separated under denaturant conditions.

### 2.2. Trimers( $\alpha\beta$ )<sub>3</sub>

Heterodimers are arranged symmetrically around a central axis (Fig. 6 A), forming ( $\alpha\beta$ )<sub>3</sub> trimers. Trimers show a discoidal structure of 120 Å in diameter, 30 Å in thickness, and a central hole of

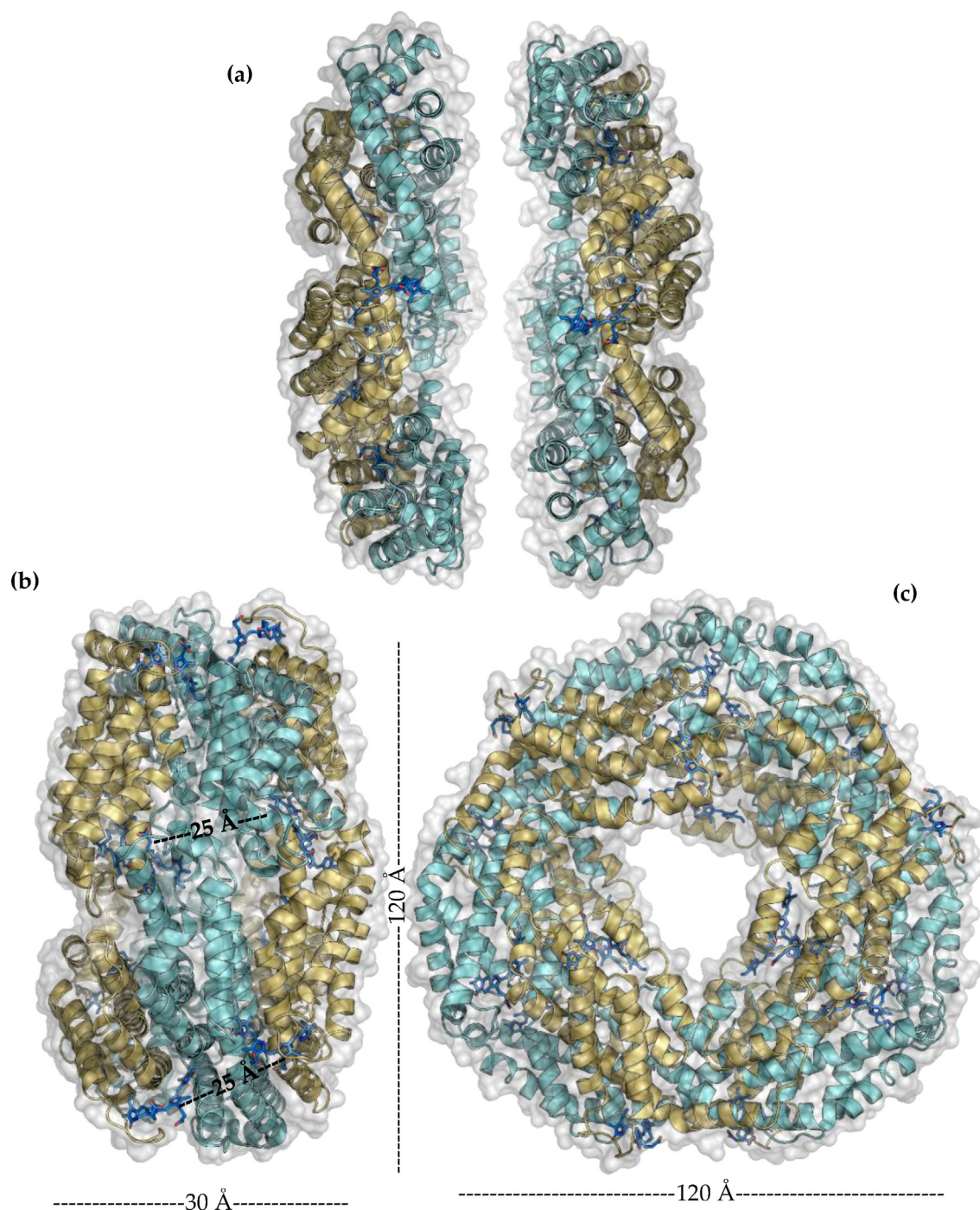


Fig. 7. Structural level of PBPs. (a) trimers ( $\alpha\beta$ )<sub>3</sub> face-to-face, (b) hexamer( $\alpha\beta$ )<sub>6</sub> side view. (c) hexamer( $\alpha\beta$ )<sub>6</sub> front view.



approximately 10 Å in diameter [13–14,25]. Within a trimer, the distance between the chromophores of two adjacent heterodimers is close to 20 Å, allowing the transfer of energy between  $\alpha$  and  $\beta$  subunits. This characteristic makes trimers the minimum functional unit of PBP and the biological unit of APC.  $(\alpha\beta)_3$  trimers have a curvature, where the concave surface is called the face and the convex surface is called back (Fig. 6 B). In the case of APC, the trimer is its functional biological assembly. The trimeric structure of APC is sensitive to temperature and chemical denaturants, which change its absorption and emission spectrum. However, both spectra recover to native state upon removal of the disturber [35–37]. Together with the above, the spectrum observed under denaturing conditions corresponds to that of the  $\alpha\beta$  heterodimer, which is not affected under the conditions tested [35]. When analyzing the contact surfaces and interactions that stabilize the trimeric structure of APC, an average area of 500 Å<sup>2</sup> with 8 hydrogen bonds and 2 contact salt bridges between each pair of heterodimers can be observed [38].

### 2.3. Hexamers $(\alpha\beta)_6$

Trimers  $(\alpha\beta)_3$  associate face-to-face (Fig. 7 a) to form hexamers  $(\alpha\beta)_6$  (Fig. 7 b and c), these being the functional biological unit of PBPs, with the exception of APC [39–42]. In the face-to-face association of the two trimers, one trimer is rotated approximately 30 degrees relative to the other [43]. The contact surface that allows the formation of hexamers is stabilized by interactions between residues of the  $\alpha$  subunits of each trimer [43]. The distance of inter-trimeric chromophores in a hexamer is approximately 25 Å. Theoretical studies based on structures [17–18] and molecular models [21] of PBPs propose energy transfers in a hexamer from one trimer to the other using the chromophores of the  $\alpha$  subunits that form the binding surface.

Hexamers and trimers of PBPs associate and form PBS functional structures, such as the rod and core [13,25,44].

### 2.4. Rods and core

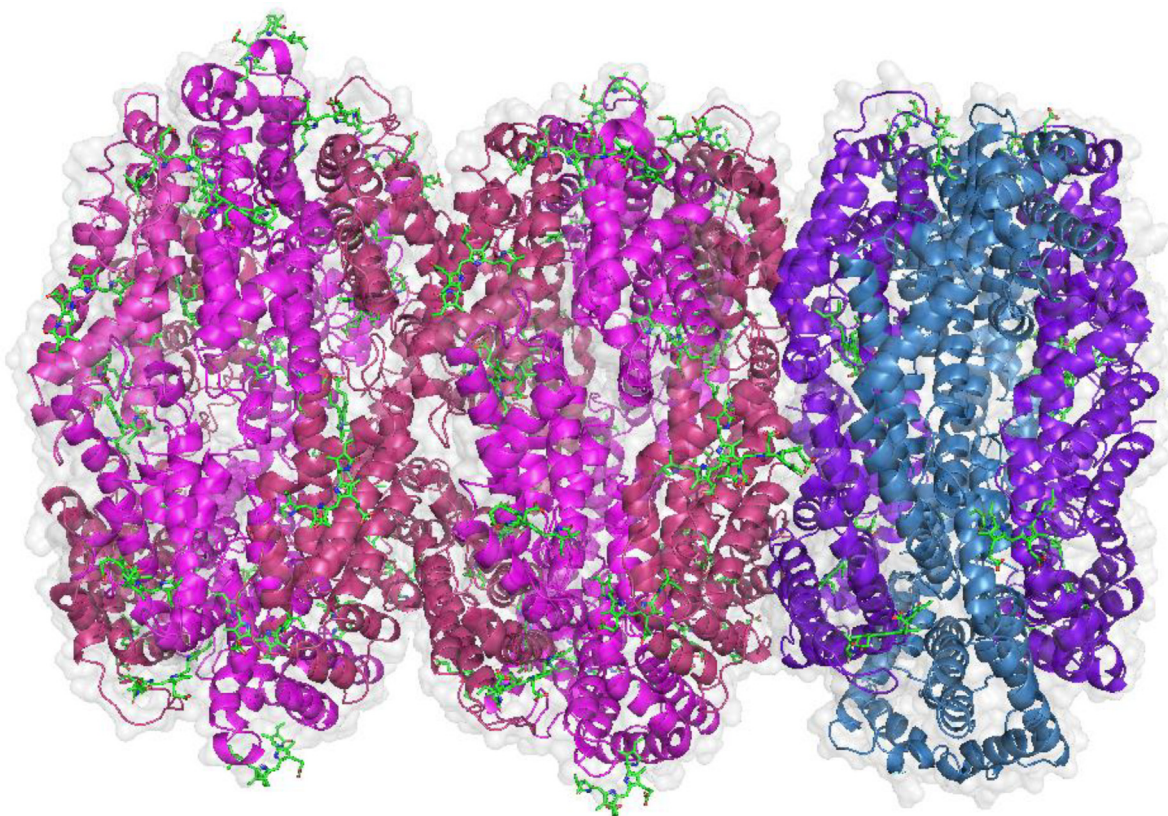
PBS rods are cylinder-shaped structures composed of multiple hexamers of PE or To form these cylinders the hexamers interact in a back-to-back using the  $\beta$  subunits to form the interaction surface PC (Fig. 8).

The core of the PBS is made up of three cylinders, each one is formed by three APC trimers. The cylinders that are in contact with the membrane have an  $\alpha$  subunit replaced by ApcD and a  $\beta$  subunit replaced by ApcF. A second  $\alpha$  unit is replaced by the globular domain (PB domain) of the Linker core-membrane (ApcE) [18,45]. ApcD, ApcF and PB domain are essential to energy transfer to the photosystems [46–49]

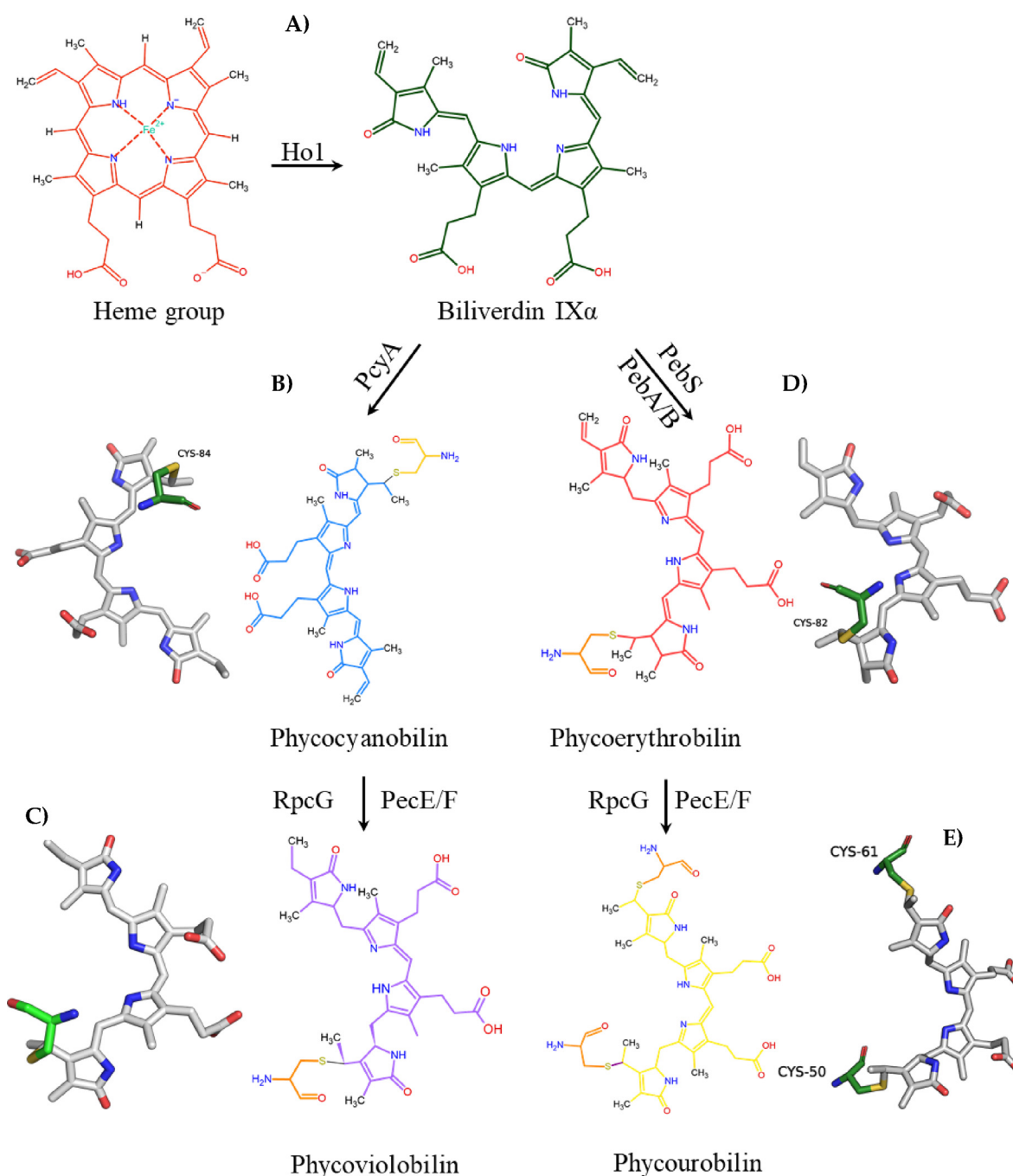
Both structures, rods and core, are essential for the function of energy transfer to the photosystems, since they allow the chromophores present in each of the subunits that make up these PBS elements to be positioned in space.

## 3. Phycobiliprotein maturation

To obtain a fully functional PBP, two independent processes are required. The first corresponds to the biosynthesis of bilins and the second stage corresponds to a process of post-translational modifications that allow the chromophores to bind covalently to the polypeptide skeleton of the PBPs.



**Fig. 8.** PBS rod. In blue  $\alpha$  subunits, of PC. In purple  $\beta$  subunits of PC. In magenta  $\alpha$  subunits of PE. In fuchsia  $\beta$  subunits of PE. In green PCB and PEB molecules. Figure made with pymol using *P. Purpureum* PBS structure (PDB id: 6KGX).



**Fig. 9.** Phycobilins biosynthesis. 2D representations are based in experimental 3D spatial orientation of phycobilins.

### 3.1. Phycobilins biosynthesis

Phycobilins are responsible of the spectroscopic properties of PBPs and understanding how these molecules are synthesised is important in the context of potential biotechnological applications of PBPs. Four types of bilins are present in red algae and cyanobacterial PBPs; namely phycocyanobilin (PCB), a blue-colored bilin with maximum absorption at 620 nm; phycoviobilobilin (PVB), that shows a violet color with a maximum absorption of 561 nm; phycoerythrobilin (PEB), which has a reddish color and has its maximum absorption at 560 nm and phycourobilin (PUB), with a yellowish color and maximum absorption at 495 nm [32,50]. The main precursor of phycobilins is Biliverdin IX $\alpha$  (BV IX $\alpha$ ), which in turn is the resulting product of the action of the enzyme heme

oxygenase 1 (HO1) [51] (Fig. 7A). BV IX $\alpha$  acts as substrate for the biosynthesis of PCB and PEB. PCB biosynthesis is mediated by the ferredoxin-dependent enzyme phycocyanobilin: ferredoxin oxidoreductase (PcyA). PcyA catalyzes a BV IX $\alpha$  reduction reaction in two steps, starting with the synthesis of 18, 18-Dihydrobiliverdin (18–18 DHVB) and subsequently 3Z-PCB [51] (Fig. 7B). On the other hand, PVB is synthesized after the isomerization of PCB mediated by the enzyme [C-phycocyanin  $\alpha$ -subunit]: Phycourobilin lyase/isomerase (RpcG) or by the action of the heterodimeric phycoerythrocyanin liase/isomerase (PecE/F) [52–53] (Fig. 7C). Finally, PEB biosynthesis starts with BV IX $\alpha$  as a precursor, which undergoes a modification by the action of the enzyme 15, 16-dihydrobiliverdin: ferredoxin oxidoreductase (PebA), forming 15, 16-dihydro biliverdin IX $\alpha$  (15, 16-DHBV). Sub-

sequently, 15,16-DHBV is transformed by the action of the enzyme Phycoerythrobilin:ferredoxin oxidoreductase (PebB) to 3Z-PEB [51]. Another route for PEB biosynthesis has been reported by Dammeyer and collaborators [54], which relies on the action of the enzyme phycoerythrobilin synthase (PebS). This enzyme also uses BV IX $\alpha$  as substrate, combining the action of PebA and PebB in one step to form PEB (Fig. 7 D).

PUB is derived from a PEB modification by isomerization mediated by phycoerythrocyanin lyase/isomerase (PecE/F) after binding to the apoprotein [55] (Fig. 7 E). Fig. 7 shows a schematic view of these phycobilins biosynthesis and their spatial orientation as obtained by x-ray diffraction.

### 3.2. Phycobilins binding to PBPs

Chromophore binding to PBPs consists of post-translational modifications mediated by specific lyase enzymes. To date, four types of bilin lyases have been described including E/F-type, S/U-type, T-type, and Y/Z-type [55–56]. These different lyases enzymes show specificity for both the chromophore as well as for binding residue in each subunit of the corresponding phycobiliprotein [57]. These enzymes ensure the binding of the correct bilin to the corresponding cysteine residue with the correct stereochemistry. In this way, one or two of the pyrrolic rings at the ends are linked through thioester bonds to cysteine residues [34].

To date, more than 1500 bilin lyases have been described, all belonging to cyanobacteria. Some of them have already been characterized at functional levels. Most cyanobacteria bilin lyases correspond to heterodimeric enzymes composed of two different subunits that, together, possess lyase activity. Each of these subunits, individually, has no activity. Three families of lyase enzymes have been described to date (E/F; CpcS/CpcU and T families) based on phylogenetic, structural, and biochemical studies, based on their respective substrates and their enzymatic activities [58].

The E/F family consists of heterodimeric lyases, with CpcE / F lyase being the first to be described in 1992 [59]. This lyase binds the PCB molecule to cysteine residue 84 of the phycocyanin  $\alpha$  subunit. Another interesting member of this family is the PecE/F lyase. This enzyme has two activities, an isomerase activity, capable of transforming PCB into PVB or PEB into PUB, and a second activity consisting of binding these to the  $\alpha$  subunit of phycocyanin or phycoerythrocyanin [52]. Kronfel and collaborators reported that a member of E-F family can bind PEB to Cys 48/59 residues of PE  $\beta$  subunit [60]. A recent study show that MpeV lyase-isomerase is

responsible of binding double linked PUB molecules to CpeB in *Synechococcus*. [61].

The CpcS/CpcU family is responsible for the binding of PCBs to cysteine 81 residues of the  $\alpha$ ,  $\beta$ ,  $\alpha$ B, and  $\beta$ 18 subunits of allophycocyanin,  $\beta$  subunit of phycocyanin, and the binding of PEB in the  $\alpha$  and  $\beta$  subunits of PE. Interestingly, the CpcS/U lyase does not bind PCBs to the phycocyanin  $\alpha$  subunit [62–63].

Finally, the T family differs from the others since they are monomeric enzymes. It has been reported that these enzymes are responsible for the binding of PCB and PEB at cysteine residue 153 of the  $\beta$  subunit of phycocyanin and phycoerythrocyanin [52]. Recent studies on the CpeT lyase suggest that this enzyme could be responsible for the binding of PEB at residues 155 of PE [64]. Table 2 shows a summary of lyase enzymes and their activity on PBPs.

Most of the data regarding bilin lyases are preserved and ordered in a database Cyanolyase [58].

### 3.3. Other post-translational modifications

The  $\beta$ -chain of PBPs has a methylation at residue asparagine 72, this modification is realized by the enzyme CpcM. The function of this change is increase energy transfer efficiency within the PBS and prevent photoinhibition [65]. Interestingly, CpcM enzyme only modify  $\beta$  subunits and not  $\alpha$  subunits, despite their similar sequence and structure.

## 4. Spectroscopic characteristics of PBPs

PBPs have been classified into large groups based on their color, highlighting four types: phycoerythrin (PE), phycoerythrocyanin (PEC), phycocyanin (PC), and allophycocyanin (APC). These, in turn, have been subclassified according to their source of origin, namely C, R, and B, from cyanobacteria, Rhodophyta algae, or algae of the bangiales order, respectively. The visible energy absorption maxima of the respective fluorescence emission maxima are 576 nm in PE, 641 nm in PC, and 661 nm in APC [13,25,44], allowing a very precise spectral coupling [66]. These spectroscopic characteristics of absorption and emission of PBPs allow a very efficient energy transfer in the PBS.

### 4.1. Phycoerythrin

Phycoerythrins (PE) have an intense pink color and the greatest diversity of chromophores among PBPs. Along with these, PE is characterized by a high quantum yield, higher than 0.9 [67]. PE is

**Table 2**

Bilin lyases family substrate specificity. PCB = phycocyanobilin. PVB = Phycoviolobilin. PEB = Phycoerythrobilin. PUB = Phycourobilin.

Lyase family	Bilin	Gene	Binding Subunit	Binding Residue
E/F	PCB	rpcA	PC- $\alpha$	Cys-84
		pecA	PEC- $\alpha$	Cys-84
	PVB	pecA	PEC- $\alpha$	Cys-84
		RpcA	PC- $\alpha$	Cys-84
S/U	PUB	pecA	PEC- $\alpha$	Cys-84
	PCB	apcA	APC- $\alpha$	Cys-81
		apcB	APC- $\beta$	Cys-81
		apcD	APC- $\alpha^B$	Cys-81
		apcF	APC- $\beta_{18}$	Cys-81
		cpcB	PC- $\beta$	Cys-81
		rpcB	PC- $\beta$	Cys-81
		pecB	PEC- $\beta$	Cys-81
		PEB	cpeA	PE- $\alpha$
	T	PCB	cpeB	PE- $\beta$
cpcB			PC- $\beta$	Cys-153
pecB			PEC- $\beta$	Cys-153
PEB		cpeB	PE- $\beta$	Cys-153
		cpcB	PC- $\beta$	Cys-153

located in the distal part of the PBS rods [17,24–25]. The  $\alpha$  subunit of PE is encoded by the *cpeA* gene and the  $\beta$  subunit by the *cpeB* gene [14]. Three types of PE have been reported in cyanobacteria or algae. C-PE is subdivided into C-PE-I and C-PE-II, R-PE and B-PE. They differ according to the number and type of chromophores that they have attached.

Type I (C-PE-I) contains 5 PEB molecules linked at residues  $\alpha$ -84,  $\alpha$ -140 or 143,  $\beta$ -84 or 82,  $\beta$ -50 or  $\beta$ -61, and  $\beta$ -155 or 159. C-PE-II has 5 PEB molecules located in the same positions as C-PE-I and has a PUB molecule bound to  $\alpha$ -75 cysteine. R-PE has 4 PEB molecules attached to residues  $\alpha$ -84,  $\alpha$ -140,  $\beta$ -84, and  $\beta$ -155, and a molecule of PUB linked to residues  $\beta$ -50 and  $\beta$ -61 respectively. In the case of red algae, B-PE has 5 PEB molecules, 2 in the  $\alpha$  subunit and 3 in the  $\beta$  subunit in residues  $\alpha$ -84,  $\alpha$ -140,  $\beta$ -84,  $\beta$ -155, and  $\beta$ -50 /  $\beta$ -61.

Spectroscopically C-PE, B-PE, and R-PE differ in their number of absorption maxima. C-PE shows only one absorption maximum at 542 nm, while R-PE and B-PE absorbance spectra show two absorption maxima; the highest at 566 nm, followed by another at 545 nm, corresponding to PEB molecules and the third maximum of lower absorbance at 495 nm, corresponding to PUB molecules present exclusively in the  $\beta$  subunit of R-PE. The fluorescence emission in the different types of PE does not present great differences with a maximum emission value at 575 nm [68–70]. Table 3 shows a summary of spectroscopic characteristics reported for different PEs.

#### 4.2. Phycoerythrocyanin

Phycoerythrocyanin (PEC) is, to date, the only phycobiliprotein exclusively present in cyanobacteria. It is a purple-bluish color phycobiliprotein, with one PVB molecule at the residue 84 of the  $\alpha$  subunit and two PCB molecules at residues 84 and 153 of the  $\beta$  subunit [68]. One of the main characteristics of phycoerythrocyanin is that its abundance is strongly related to the intensity and type of light during its growth, increasing significantly in low light conditions with green light. [71]. Their presence in PBSs is not exclusive to PE, although they can absorb similar areas of the visible light spectrum. [68]. The maximum absorbance of phycoerythrocyanin is at 570 nm with a shoulder at 590 nm. Its fluorescence emission has its maximum at 625 nm [68]. Table 4 shows the spectroscopic characteristics phycoerythrocyanin.

#### 4.3. Phycocyanin

Phycocyanin (PC), an intense, blue-colored protein, is found in the proximal part of the rods of PBS. Unlike PE, PC is always present in cyanobacteria and red algae PC. Two types of phycocyanins have

been recognized to date; PC present in red algae and cyanobacteria, whose expression is constitutive and come from the products of *cpcA* and *cpcB* genes, corresponding to the  $\alpha$  and  $\beta$  subunits of phycocyanin, respectively. The second type of phycocyanins are those present only in cyanobacteria and have an inducible character through a chromatic adaptation system. These are the product of *cpcA2* and *cpcB2* genes [72].

PC is also classified according to their spectroscopic properties [69]. C-PC, present in most cyanobacteria, has PCB molecules attached to cysteine residues at position 84 in the  $\alpha$  subunit and cysteines 84 and 155 of the  $\beta$  subunit. Its absorption spectrum shows a single absorption maximum at 620 nm and an emission maximum at 640 nm [25,50]. R-PC has different chromophores attached, depending on the origin of the phycobiliprotein: R-PC-I is the most abundant, present in red algae and it was the first to be characterized spectroscopically. This protein has a PCB molecule attached to the Cis residues –84 of the  $\alpha$  and  $\beta$  subunit and a PEB molecule attached to the Cys-155 residue of the  $\beta$  subunit. The absorption spectrum of this type of PC shows two absorption maxima, the first in less than 555 nm, attributable to a PEB molecule and a second higher maximum, associated with PCB molecules, at 619 nm. This protein has an emission maximum of 640 nm [25,73].

R-PC-II was the first cyanobacterial PC reported to possess a PEB molecule. This type of phycocyanin has a PEB molecule linked to cysteine 84 in its  $\alpha$  subunit, while the  $\beta$  subunit has a PCB molecule linked to cysteine 84 and a PEB molecule to cysteine 155. Its absorption spectrum shows three peaks at 533, 545, and 615 nm. Its fluorescence emission is 646 nm [25,50].

R-PC-III has two PCB molecules and one PEB, but unlike R-PC-I and R-PC-II, the PCB molecules are both at residues 84 and 153 of the  $\beta$  subunit and the molecule of PEB at residue 84 of the  $\alpha$  subunit. It has two absorption maxima, one at 560 nm and the second of less intensity at 603 nm. The emission maximum of this phycocyanin is at 648 nm [50].

R-PC-IV differs from other types by having a PUB molecule attached at residue 84 of the  $\alpha$  subunit and two PCB molecules at residues 84 and 155 of the  $\beta$  subunit. Its absorption spectrum shows two maxima, one at 490 nm and the other at 592 nm. The emission maximum of this protein is at 644 nm [25].

In 2009, Blot and collaborators. described a fifth type of phycocyanin, R-PC-V. This protein is characterized by having three different types of PUB chromophores at residue 84 of the  $\alpha$  subunit, a PCB molecule at residue 82, and a PEB molecule at residue 153 of the  $\beta$  subunit.

R-PC-V has three absorption maxima, at 495 nm, 540 nm, and 590 nm. Fluorescence emission at 640 nm [52]. Table 5 shows a

**Table 3**  
Spectroscopic properties of different phycoerythrins. PEB = Phycoerythrobin. PUB = Phycouobilin.

Protein	Binding Residue						$\lambda_{Abs_{Max}}$ (nm)	$\lambda_{Em_{Max}}$ (nm)
	Cys $\alpha$ -75*	Cys $\alpha$ -84	Cys $\alpha$ -143	Cys $\beta$ -[50 61]	Cys $\beta$ -84	Cys $\beta$ -155		
C-PE	C-PE I	PEB	PEB	PEB	PEB	PEB	565	575
	C-PE II	PUB	PEB	PEB	PUB	PEB	565	575
R-PE		PEB	PEB	PUB	PEB	PEB	495, 545, 566	574
B-PE		PEB	PEB	PEB	PEB	PEB	545, 565	576

**Table 4**  
Spectroscopic properties of different phycoerythrocyanin. PCB = phycocyanobilin. PVB = Phycoviolobilin.

Protein	Binding Residue			$\lambda_{Abs_{Max}}$ (nm)	$\lambda_{Em_{Max}}$ (nm)
	Cys $\alpha$ -84	Cys $\beta$ -84	Cys $\beta$ -155		
PEC	PVB	PCB	PCB	570	625

summary of the spectroscopic properties of the different phycocyanins.

#### 4.4. Allophycocyanin

Allophycocyanin (APC) has a bright turquoise color and is exclusively found as part of the PBS core. Spectroscopically, it is the simplest phycobiliprotein since it only has one PCB molecule at residue 81 of the  $\alpha$  and  $\beta$  subunit. The biological unit of allophycocyanin is trimeric. APC differs from other PBPs as it has 2 codifying genes for the  $\alpha$  subunit, called *apcA* and *apcD*, whose gene products are known as  $\alpha$  subunit and  $\alpha^B$  subunit, respectively. It also has 2 codifying genes for the  $\beta$  subunit, called *apcB* and *apcF*, responsible for the  $\beta$  and  $\beta^B$  [18] subunits, respectively. Both *ApcD* and *ApcF* have lower abundance and replace the  $\alpha$  and  $\beta$  subunits in different trimers that form the core of the phycobilisome.

It has been demonstrated from the complete sequencing of the chloroplastidial genome of *Gracilaria tenuistipitata* var *liui* [74], *Agarophyton chilense* [75], and *Gloeobacter violaceus* [76], that the genes of allophycocyanin are encoded in the *apcEABC* cluster, however, the *apcD* and *apcF* genes are located isolated in different areas of the chloroplastid genome [74].

Trimers composed only by  $\alpha$  and  $\beta$  form an  $(\alpha\beta)_3$  complex, which are the most common trimers and have an absorption maximum of 650 nm and a fluorescence emission maximum of 660 nm. It has been reported an allophycocyanin trimer  $(\alpha\beta)_3$  that also includes a linker protein know as linker core (Lc) of 8.9 kDa, the presence of this protein produces an spectroscopic change in 2 nm in the absorption maximum and 2 nm in the emission maximum [25].

The replacement of an  $\alpha$  subunit by the  $\alpha^B$  subunit within a trimer  $(\alpha^B\alpha_2\beta_3)$  shows spectroscopic properties that differ from trimer  $(\alpha\beta)_3$ , with a shift of absorption maxima at 654 nm and emission maxima at 679 nm [22,25].

The trimers that have the  $\beta$  [18] subunit differ from others since, in addition to replacing a  $\beta$  subunit, there is a change of an  $\alpha$  subunit for the PB domain at the core-membrane linker (Lcm) [22] forming a  $(\alpha_2 L_{cm} \beta_2 \beta^{18})$  trimer. The  $\beta$  [18] subunit is also distinguished from others by its size, since it is made up of 169 residues, unlike the other three allophycocyanin subunits which are made up of 161 residues. This type of trimer has an absorption maximum at 654 nm and an emission maximum at 670 nm [25].

The absence of the  $\alpha^B$  and  $\beta$  [18] subunits in cyanobacteria has shown to decrease the efficiency of energy transfer from the phy-

cobilisome to photosystem I and photosystem II, respectively [48,49,77–78]. Table 6 shows a summary of the spectroscopic properties of the different types of allophycocyanin trimers.

In summary, the information reviewed shows that the different PBPs share a series of general characteristics, such as their structure and function. The presence of these proteins in the context of the PBS has allowed different organisms to be able to populate and survive in adverse environmental conditions, giving an advantage to the species that possess them. However, PBPs have shown more activities than just energy capture. For example, in cases of nitrogen deprivation, PBPs are used as source of nitrogen, allowing the synthesis of other proteins [79]. The truncation of the PBS in *Synechocystis* sp. *PCC 6803* shows alteration in the utilization and regulation of iron and bicarbonate, also has implications in the proteomic profile and cellular membrane associated functions [80–81]. which suggest that PBS and PBPs have more functions than be only a light harvest complex

The following section reviews multiple industrial applications of PBPs associated with its biotechnological processes.

### 5. Biotechnological perspectives of PBPs

PBPs has important biological properties for humans and animals that motivate the development of new biotechnological products and processes [82–83]. Recently, several studies have reported the bioactivities of the PBPs, which can be summarized in anti-oxidant, anti-inflammatory, anti-metabolic diseases, anti-cancer, anti-neurodegenerative, and anti-pathogenic microorganisms. The details of the different biological activities of the PBPs are extensively reviewed in previous works [84–88]. In this section we will briefly see the biological activities of the PBPs present in publications of the last two years (Table 8).

#### 5.1. Biological activities

##### 5.1.1. Anti-oxidant activity

Cellular oxidative stress can be prevent by PBP, specifically, PC (Table 8). PC can inhibit the oxidative activity of various radicals such as peroxyl, hydroxyl, and superoxide, inhibiting lipid peroxidation [89–90]. Together with the above, PC can inhibit the action of peroxynitrite radicals, thus reducing DNA damage [91].

Recently, the anti-oxidant activity of C-PC was reported in different application areas as for the reduction of acute liver oxidative

**Table 5**  
Spectroscopic properties of different phycocyanins. PCB = phycocyanobilin. PEB = Phycoerythrobilin. PUB = Phycourobilin.

Protein	Binding Residue			$\lambda_{Abs\ Max}$ (nm)	$\lambda_{Em\ Max}$ (nm)
	Cys $\alpha$ -84	Cys $\beta$ -84	Cys $\beta$ -155		
C-PC		PCB	PCB	620	640
R-PC	R-PC-I	PCB	PCB	555, 619	640
	R-PC-II	PEB	PCB	533, 545, 615	646
	R-PC-III	PEB	PCB	560, 603	648
	R-PC-IV	PUB	PCB	490, 592	644
	R-PC-V	PUB	PCB	495, 540, 590	640

**Table 6**  
Spectroscopic properties of different allophycocyanins. PCB = phycocyanobilin.

Trimer	Binding Residue		$\lambda_A\ max$ (nm)	$\lambda_E\ max$ (nm)
	Cys $\alpha$ -84	Cys $\beta$ -84		
$(\alpha\beta)_3$	PCB	PCB	651	660
$(\alpha\beta)_3 + Lc$	PCB	PCB	653	662
$\alpha^B\alpha_2\beta_3$	PCB	PCB	654	679
$\alpha_2\beta_2\beta^{18} + Lcm$	PCB	PCB	654	670

**Table 8**  
Biological effect of PBP and its proposed application. Studies reported between 2019 and 2021.

PBP	Biological activity	Source of PBP / Biological Effect	Proposed Application	Reference
PC	Antioxidant	<i>Arthrospira maxima</i> / PCB prevents alterations in oxidative stress markers, antioxidant enzymes, and caspase 9 activities	Nephro-protective action on acute kidney injury caused by mercury	[93]
		<i>Spirulina</i> sp. / Attenuate radiation-induced oxidative stress damage in liver by activating Nrf2/ HO-1 signaling pathway and reduce DNA damage.	Protective effect on hepatic damage induced by X-ray	[92]
		<i>Spirulina</i> sp. / Dietary supplementation reduces the oxidative stress in liver and kidney induced by a diet enriched with lipid peroxides in Wistar strain rats.	Diminish the risk of pathologies related to oxidative stress due to high oxidize oil consumption.	[115]
		<i>Spirulina</i> sp. / Lengthening delivery of PC, encapsulated in poly(ethylene glycol)-b-(poly(l-glutamic acid)-g-polyethylenimine nano-carrier, through the abdominal subcutaneous injection in rats.	Attenuation of hepatic ischemia/reperfusion-induced pancreatic islet injury.	[116]
		<i>Phormidium versicolor</i> / Prevents cadmium-induced elevation of ALAT, ASAT and bilirubin levels in rats. Enhance the levels of antioxidant enzymes.	Prevention action against hepatotoxicity caused by cadmium.	[117]
	Anti-inflammatory	<i>Spirulina</i> sp. / Inhibits of albumin denaturation, anti-proteinase, hypotonicity-induced haemolysis and anti-lipoxygenase activities.	Potential drug development.	[95]
		<i>Spirulina platensis</i> / Reduce the micturition frequency and bladder inflammation in mice with cyclophosphamide-induced cystitis by inhibiting COX-2 and prostaglandin E receptor 4.	Countermeasure for the cyclophosphamide-Induced Cystitis anticancer chemotherapy	[118]
		<i>Spirulina platensis</i> / Inhibits COX-2 expression during the radiation therapy of the colon cancer cell lines and in normal colonic cells.	Countermeasure reduction by C-PC treatment during colonic cancer radiation therapy.	[94]
		<i>Spirulina platensis</i> / Attenuates the pulmonary fibrosis by inhibition of the production of interleukin-1 beta, tumor necrosis factor- $\alpha$ , and lipopolysaccharide. Increases the intestinal bacterial diversity, richness, and reduces pro-inflammatory bacteria.	Idiopathic pulmonary fibrosis treatment	[119]
		<i>Arthrospira platensis</i> / APC and C-PC tryptic peptides inhibit enzyme DPP-IV activity <i>in vitro</i> , therefore, acting as hypoglycemic peptides.	Nutraceuticals or functional foods for the management of type 2 diabetes.	[98]
PC	Anti-metabolic diseases	<i>Spirulina platensis</i> / Reduce lipid accumulation in the steatosis LO2 cells and in the liver of NASH mice. Improve the antioxidant capacity of liver by activating AMPK pathway of hepatocytes.	Nutraceuticals and therapeutics of non-alcoholic fatty liver disease.	[45]
		<i>Spirulina platensis</i> / Inhibits $\alpha$ -amylase and $\beta$ -glucosidase enzymes activity.	Potential drug development for diabetes type 2 treatment.	[95]
		<i>Limnothrix</i> sp. / Regulates both anti- and pro-apoptotic genes by increasing levels of Bax, Apaf-1 and activates caspase-8, caspase-9, and caspase-3. Decreasing expression of Bcl-2, Mcl-1, and survivin . In combination with Topotecan, increase expression of the death receptor FAS and cleaved PARP.	Prostate cancer cells (LNCaP) treatment with reduction of conventional chemotherapeutic agents such as Topotecan.	[102]
	Anti-cancer	<i>Spirulina platensis</i> / Anti-proliferative effect against HepG-2 cell lines.	Potential drug development for cancer treatment.	[95]
		<i>Limnothrix</i> sp. KNUA002 / The use of PC before cisplatin anti-cancer chemotherapy inhibits apoptosis and protected mitochondrial function by preventing ROS accumulation in cisplatin-treated House Ear Institute-Organ of Corti 1 cells. Maintains Bax and Bcl-2 levels close to untreated control.	PC protection of cells against cisplatin-induced cytotoxicity as an anti-cancer therapy countermeasure	[120]
		<i>Spirulina</i> sp. / Ameliorates the radiation-induced intestinal injury and regulates the effect on gut microbiota by TLR4/Myd88/NF- $\kappa$ B pathway	Countermeasure for the radiation-induced gastrointestinal syndrome	[121]
		<i>Spirulina platensis</i> / Chlorophyllin-PC mixture plus diode laser irradiation reduced the ex-vivo cariogenic biofilm of <i>Streptococcus mutans</i> by increasing its ROS generation.	Antimicrobial photodynamic therapy for caries treatment as an ancillary approach	[112]
		<i>Spirulina</i> sp. / Induce apoptosis, suppressed the growth of NSCLC cells by down regulation of TIRAP/NF- $\kappa$ B activity	Non-small cell lung cancer treatment.	[104]
		<i>Spirulina platensis</i> / Anti-proliferative and anti-migratory function by the reduction of RIPK1/NF- $\kappa$ B activity in the NSCLC cells.	Non-small cell lung cancer treatment.	[105]
		<i>Spirulina platensis</i> / Anti-proliferation of esophageal squamous cell carcinoma by cell cycle arrest, induction apoptosis and suppression invasion ability. Elevation of Bax, PARP, and cleaved-caspase-3 protein, but reduced cyclin D1, CDK4, Bcl-2, MMP-2, and MMP-9 expression.	Esophageal squamous cell carcinoma treatment	[122]
PC	Anti-cancer	<i>Spirulina</i> sp. / The C-PC/CMC-CD59sp nanoparticles up-regulates the expression of p21, and then down-regulates the expressions of Cyclin D1 and CDK4 in BALB/c nude mice. Up-regulates the expression of cleave caspase-3, down-regulates the expression of bcl-2, and inhibit MMP-2 protein expression.	New drug delivery system with antitumor effects containing C-PC as main bioactive molecule.	[123]
		<i>Spirulina platensis</i> LEB 52 / In multiple drug resistance phenotype of erythroleukemic cells, C-PC modulates the expression of COX2 and ABCB1 for the K562-Lucena cells in a ROS-dependent manner and the expression of ALOX5 for the FEPS cells in a ROS-independent manner.	Erythroleukemic cells multiple drug resistance (MDR) phenotype treatment.	[124]
		<i>Galdieria sulphuraria</i> / Aqueous extract containing C-PC has antioxidant activity and exert cytotoxic activity in the human adenocarcinoma A549 cells.	Anticancer candidate for therapeutic treatments	[125]
	Anti-neuro-degenerative	<i>Spirulina platensis</i> / PCB reduces brain injury in PC12 neuronal cells after endothelin-1 induced focal cerebral ischaemia in Wistar rats. PCB restored the myelin basic protein and CNPase enzyme expression levels in ischaemic rats.	PCB as a therapeutic pharmacological alternative for ischaemic stroke patients.	[126]
		<i>Spirulina</i> sp. / Prevents streptozotocin-induced increase activity of hippocampal cholinesterases, BAX, and the levels of BCL-2 and ChAT.	Therapeutic agent in managing Alzheimer's Disease	[110]

(continued on next page)

Table 8 (continued)

PBP	Biological activity	Source of PBP / Biological Effect	Proposed Application	Reference
		Also, attenuates neuro-inflammation and dysfunctional insulin signaling. <i>Spirulina</i> sp. / Inhibits A53T $\alpha$ -synuclein and A $\beta$ 40/42 fibril formation, but do not inhibit the reduction-induced amorphous aggregation of ADH and heat-induced aggregation of catalase.	Development of therapeutics to prevent Alzheimer's and/or Parkinson's disease.	[109]
	Anti-micro-organism	<i>Spirulina</i> sp. / PCB inhibits the main protease and papain-like protease activities from coronaviruses (CoV) <i>in vitro</i> . <i>In silico</i> , showed inhibitory activity using other phycobilins such as PUB, PEB, and PVB	PCB as broad-spectrum antiviral drugs against coronaviruses.	[114]
		<i>Spirulina</i> sp. / Bacterial abundance and diversity increase in mice intestinal. Decrease intestinal permeability and increases barrier function.	Basis for a positive modulation of intestinal microbes by treatment with PC.	[127]
		<i>Spirulina</i> sp. / Reduce the culture viability of <i>E. faecalis</i> in human premolar teeth by increasing the bacterial internal ROS.	Adjunct therapy in endodontic infection treatment	[128]
APC	Antioxidant	<i>Phormidium</i> sp. / APC increase anti-aging effect in worm model by improving physiological markers. Moderates the expression of human A $\beta$ 1–42.	The use of APC as drug development for age-related disorders.	[129]
	Anti-inflammatory	<i>Pyropia pseudolinearis</i> / Peptides produced from the water-soluble proteins (riched in PE, PC, APC, and Rubisco) had inhibitory effect on the angiotensin-I-converting enzyme	Prevention of cardiovascular diseases and high blood pressure.	[130]
PE	Antioxidant	<i>Pyropia yezeensis</i> / PE had <i>in vitro</i> antioxidant activity and cytotoxicity against HepG2 cells (hepatocellular carcinoma cells)	Prevention of the hepatocellular carcinoma cells.	[131]
		<i>Phormidium persicinum</i> / C-PE reduce expression of nephrin and podocin in a mouse model of HgCl <sub>2</sub> -induced acute kidney injury. Reduced activation of IRE1 $\alpha$ pathway and avoids caspase-mediated cell death.	Prevention of HgCl <sub>2</sub> -induced acute kidney injury.	[132]
	Anti-inflammatory	<i>Colaconema</i> sp. / Enhance prophenoloxidase and phagocytosis activity but inhibits ROS in hemocytes from whiteleg shrimp. <i>In vivo</i> , PE induce immune genes and enhance the innate immune parameters.	Potential immunomodulator in shrimp culture to prevent the <i>Vibrio parahaemolyticus</i> or white spot syndrome virus	[133]
	Anti-metabolic diseases	<i>Porphyridium purpureum</i> / Peptide from PE reduce the foam cell formation, intracellular lipid accumulation and the secretion of TNF- $\alpha$ and IL-6 in RAW 264.7 cell line by targeting the scavenging receptors CD36, SRA1, and Map Kinase p38.	Prevention atherogenic foam cell formation.	[134]
	Anti-cancer	Inhibits growth and induce apoptosis of SKOV-3 cells. The ROS/JNK/Bcl-2 signaling pathway, upregulation of JNK, GADD45A, RAD23, and downregulation of XBP1 and OS9 are critical in PE -induced apoptosis in this cell.	PE as a potential treatment for human ovarian cancer.	[135]
		R-PE has modulated the innate and adaptive immune systems through TLR4/NF- $\kappa$ B mediated CD4+ T cell activation and differentiation in a hydrocortisone immune-suppressive model.	Immuno-modulator during long-term high-dose glucocorticoids therapy for autoimmune diseases and cancer	[96]
PE	Anti-neurodegenerative	<i>Porphyra haitanensis</i> / C-PE inhibits the precursor protein of BACE1, therefore, reduce accumulation of amyloid- $\beta$ precursor protein.	Therapeutic drug for complement Alzheimer's disease treatment	[111]
	Anti-microorganism	Recombinant PE / R-PE increase the abundance of beneficial bacteria in the intestinal flora and reduce the detrimental bacteria from H22-bearing mice.	Gut microbiota modulation for prevention of resistance diseases and nutritional in animals.	[136]

damage caused by X-ray (*in vivo* study) [92] or for prevention of the oxidative stress in acute kidney damage caused by HgCl<sub>2</sub> [93].

### 5.1.2. Anti-inflammatory activity

PBP have been reported to have a series of anti-inflammatory activities, especially phycocyanin (Table 8). In general, the anti-inflammatory activity is related to the inhibition of the COX-2 activity, myeloperoxidase activity, suppression of apoptosis and reduction of autoimmune response [84,87]. In addition, to reduce patient radiation therapy countermeasure, C-PC was proposed as radiosensitizing due to the inhibition of COX-2 expression during the colon cancer radiation therapy [94]. Recently, anti-inflammatory effects such as *in vitro* inhibition of albumin denaturation, anti-proteinase, hypotonicity-induced haemolysis and anti-lipoxygenase activities were determined using phycocyanin [95]. On the other hand, immuno-modulatory activity of R-PE in the innate and adaptive immune systems via TLR4/NF- $\kappa$ B-dependent immunocyte differentiation was identify in a hydrocortisone (HC)-induced immunosuppressive model [96].

### 5.1.3. Anti-metabolic disease activity

PBP, mainly PCB and PC (Table 8), have been reported with anti-diabetes activity due to their inhibition of NADPH oxidase and protective effect against hLEC (human lymphatic endothelial cells) apoptosis [84]. Recently, PC is a potential candidate for anti-

diabetic (type 2) natural therapeutic agents due to the *in vitro* inhibition of carbohydrate-metabolisms ( $\alpha$ -amylase and  $\alpha$ -glucosidase) and dipeptidyl peptidase IV enzymes (PC and its tryptic peptides) [95,97–98]. Anti-obesity activity was identified for PC by inhibition of the pancreatic lipase activity [99]. In addition, C-PC reduce the lipid accumulation in the steatosis L02 cells and liver of non-alcoholic steatohepatitis (NASH) mice, and improve the antioxidant capacity of liver [45].

### 5.1.4. Anti-cancer activity

PBP anti-cancer activity has been identify in several cancer types and/or tumor cells [84,87,100–101] (Table 8). PC presents the highest activity by altering the growth of various tumor cell lines at different levels of their molecular mechanisms. Its anti-cancer effect on tumor cell (*in vitro* and/or *in vivo* studies) has been reported on breast, cervical, prostate, liver, lung, pancreatic, colon, leukemia and bone marrow cancers, where the cell cycle arrest/reduced proliferation, reduced tumor cell migration/invasion, and apoptosis/necrosis are the principal molecular mechanisms [100,102]. Recently, MacCarty and collaborators [103] hypothesized the ability of PBP to prevent cancer cachexia by reduction of TLR4 signaling in skeletal muscles and the effect of phycocyanobilin on pancreatic cancer by inhibition of mitochondrial ROS. In addition, C-PC suppresses the *in vitro* proliferation and migration of non-small-cell lung cancer cells through the reduction

of RIPK1/NF- $\kappa$ B and TIRAP/NF- $\kappa$ B activity [104–105]. In general, PC had almost no or slight proliferative effects on cells from normal tissue, and high level concentration does not affect normal cell viability [106]. Nevertheless, the main mechanisms of action and its cellular targets have not yet been defined [101].

#### 5.1.5. Anti-neurodegenerative activity

Parkinson's and Alzheimer's disease (Table 8), which are mediated by the misfolding and aggregation of proteins such as  $\alpha$ -synuclein ( $\alpha$ S) and amyloid- $\beta$  ( $A\beta$ ), respectively [107], were inhibited by C-PC [108], but was ineffective in inhibiting the reduction-induced amorphous aggregation of ADH and heat-induced aggregation of catalase [109]. In addition, intracerebroventricular cognitive decline in the Alzheimer's disease [110]. PE also was proposed as a putative therapeutic drug for the Alzheimer's disease due to the inhibition of the *beta*-site amyloid precursor protein cleaving enzyme-1 (BACE1) [111]. Future studies should focus on the understanding of the mechanisms responsible for the formation and inhibition of these protein aggregates and potential therapeutic development.

#### 5.1.6. Anti-microorganism activity

PBP with anti-bacterial, anti-fungal and anti-virus activities were identified, mainly for PC by growth inhibition of selected microorganism. Recently (Table 8), antimicrobial photodynamic therapy using a diode laser (DL) plus chlorophyllin-phycoerythrin mixture as photosensitizer reduced dental caries produced by *Streptococcus mutans* [112]. This indicates the broad range of applications in the field of antibacterials. Anti-fungal activity of C-PC was also identified by the inhibition of cytopathic effects [113]. Anti-virus activity was reported for PCB, PUB, PEB, and PVB, which inhibits the SARS-CoV-2 and others coronavirus (CoV) proteases [114].

### 5.2. Biotechnological applications

The PBPs are used in several industrial sectors [137–138]. Among the different commercial applications, they can be found as food coloring additives, and in cosmetic products. Also, they can be found in clinical or research laboratories as fluorophores [139]. PBPs are used as natural food colorants over synthetic dyes which exhibit carcinogenicity. *S. platensis* and *Porphyridium aeruginosum* extracted PE is utilized as confectionary colors, gelatin desserts, and cosmetic products. Also, PC is a pharmaceutically active phycopigment due to its antioxidant, neuroprotective, and hepatoprotective properties [139].

#### 5.2.1. Production of PBPs from natural sources

Appropriate optimization of growth conditions is key for the development of PBPs industry and its further processing. Because the production and growth of metabolites in cyanobacteria depend not only on biotic factors but also on abiotic factors (light, temperature, nutrient concentration, pH, salinity and chemical composition of the medium can modify the metabolism of the organism) [84]. The culture conditions to increase the biomass production of different microalgae, such as *Spirulina platensis*, is one of the tools that are used experimentally and industrially for the accumulation of PBPs. Among the factors that have been used in these studies is the obtaining of biomass rich in PBPs under photo, hetero and mixotrophic conditions [140]. The optimization of photobioreactors, medium composition, and culture environments are the major variables researchers use for rapid biomass accumulation and high production [140–141].

The other main source of obtaining PBPs, apart from cyanobacteria, are red algae. These can be micro or macro algae. For years, various efforts have been made to cultivate them and promote

the production of PBPs. In 2021 Rula and collaborators testing three factors (Light intensity, water motion and poblational density) to enhance growth and the PE production of *Halymenia durvillei* [142]. Another perspective in search of increasing the production of PBP in red algae was used by Souza and collaborators, who used biostimulants, derived of brown algae, to increase the amount of PE, PC and APC of *Gracilaria caudata* and *Laurencia catarinensis* with considerable success [143]. Not only the amount of light has been proven to increase the production of PBPs, but also the spectral composition and its effect on increasing the production of PBPs in the algae *Halyenia floresii* [144]. Similar approaches have been made for the macroalga *Colaconema sp* and *Porphyridium purpureum* [145–147]. Achieving the balance between the increase in biomass and the productivity of PBPs is the current challenge, both for cyanobacteria and for red algae.

#### 5.2.2. Recombinant PBPs

Another alternative for the production of PBPs is the use of bacteria such as *E. coli* for their biosynthesis via heterolog expression. This approximation has been succeeded by genetic engineering techniques [31,50,56,148–149]. Two processes are involved in the biosynthesis of PBPs: 1) the synthesis of apoproteins and phycobilins and 2) the binding of phycobilins to apoproteins by enzymatic catalysis [150–151].

After being synthesized in *E. coli*, phycobilins need to bind to the correct site of the apoprotein. In recombinant PBPs, the linkage between a phycobilin and apoprotein is formed with higher efficiency and correctness when catalyzed by a lyase. Therefore, the use of PBP lyases is the key to efficiently synthesize recombinant PBPs *in vitro* [86].

A current common problem with recombinantly synthesized PBPs is that the recombinant only binds to one chromophore molecule, and its Stokes shift is small, usually 10–25 nm. In *E. coli* genes were introduced as phycobiliprotein lyase genes *cpcS* and *cpcT*, the phycocyanin  $\beta$  subunit gene *cpcB*, the heme oxidase *Ho1* gene, and the phycoerythrobilin reductase *pebS*. This methodology constructs a synthesis pathway of the phycobiliprotein fluorescent protein and obtains the recombinant fluorescent protein with the large Stokes shift [152].

By molecular design, several improved functions could be obtained with multiples applications. For example, a fusion protein has been developed between phycocyanin  $\alpha$ -subunit CPCA gene and Light-Harvesting Complex LHC-II gene in *E. coli*. This new product displays a wide-range absorption spectrum, a great feature for photosensitizers, used as a natural dye in solar panels [153].

#### 5.2.3. Extraction of PBPs

Technology approaches are available for the industrial extraction of PBPs from algal sources. However, the selection of methodology depends on factors such as organism composition, stability, and cell wall resistance [139]. In addition to the yield of PBP biosynthesis, the recovery of PBPs from biomass should also be considered. Different grades of purity are required depending on the intended application of the PBP. The purity of PBPs is usually expressed by the ratio  $A_{\max}/A_{280}$  [86]. For foods and cosmetics, the reported purity values are between 0.56 and 4.4.

Extraction processes can be used to obtain the released pigments from ruptured biomass (organic solvent extraction, pressurised solvent extraction, ionic liquid extraction, and supercritical carbon dioxide extraction [139]).

Several technologies are available for rupturing microalgal cell walls to release intracellular pigments mechanical treatments (high-pressure homogenization (HPH), bead milling, ultrasonication, pulse electric field, freezing-thawing process, osmotic shock, and microwave), enzymatic treatments, and chemical treatments (e.g., phosphate buffer). However, is species-specific and the cell



wall structure [154]. Comparing the different treatments, one can find clear advantages and disadvantages for production. Mechanical methods are not selective and consume considerable amounts of energy, however, they are non-toxic, fast, and suitable for large scale production. In contrast, chemical and enzymatic treatments are described as less suitable for scaling-up due to their high cost, low stability, time consumption, and toxicity [141].

#### 5.2.4. Purification of PBPs

It is possible to purify the PBPs by precipitation, sugar gradient centrifugation, and chromatographic techniques [84]. Different amounts of ammonium sulfate precipitation activated carbon and chitosan precipitation are used. Among them are aqueous two-phase extraction with polyethylene glycol, concentration with ultrafiltration or tangential flow ultrafiltration (30–50 kDa). Then, the extract can be purified by a chromatographic column due to differences in the colors and polarity of the pigments or their size (anion exchange chromatography with Q-Sepharose column, gel permeation chromatography with Sephadex G-150 column, and anionic chromatography with diethylamino ethanol cellulose).

Previous studies investigated the recovery of PBP from microalgal and cyanobacterial biomass [155], developed a rapid method for the extraction and quantification of cryptophytic PE from *Rhodomonas salina*, and the cell disruption and extraction conditions were freezing and phosphate buffer. Cryptophytes do not have a cell wall; they possess a fragile periplast beneath the plasma membrane that can be easily ruptured by lyophilization action. A simple filtration was required to obtain a maximum yield/recovery of 8.04 µg PE/cell. [155].

Another method is that of Rodrigues and collaborators [156] who reported the application of protic ionic liquids in the microwave-assisted extraction (MAE) of PBPs from *Arthrospira platensis*. They worked focusing on APC, PC and PE. The extraction was combined with the use of protic ionic liquids, 2-hydroxyethyl ammonium acetate (2-HEAA), and 2-hydroxyethyl ammonium formate (2-HEAF) (1:1) The purity obtained for PC, APC and PE was 1.22, 1.03, and 0.71, respectively [156].

Thus far, it has been reported that effective separation and purification methods to obtain higher purity PBPs are still lacking, which has greatly restricted their applications [84,86].

The cell disruption, extraction, and purification process have been developed by a combination of the above-mentioned methods. For example, in *Spirulina*, a method was developed where samples were placed in a freezer at –28 degrees C. Then, they were three times freeze-thawed, the biomass was homogenized for approximately 5 min to break up the cells, and later centrifuged [157].

Chitosan was used for the purification of these extracts at 2% and ion-exchange chromatography [157] showed high purity of PBPs. Another solution to obtain a high purity product is a powder from algae mix *Porphyra yezoensis*, red algae, cyanobacteria, and *Spirulina platensis*, which are triturated, precipitated with ammonium sulphate and hydrolyzate with trypsin [158]. This strategy generates phycobiliprotein polypeptides that are easy to digest and absorb, destined for the medical, cosmetic, and food industry.

#### 5.2.5. Encapsulation of phycobiliproteins

The encapsulation of PBP improves its thermal protection and preserves its active properties. In this context, the electrospraying method showed high encapsulation efficiency of phycocyanin [159], with especial emphasis on the food industry. In addition, other techniques have been applied in the last years for the microencapsulation of phycocyanin [160–164] in order to increase its stability. Nevertheless, a specific delivery of the PBPs should ensure its bioactivity in the target cells or tissues, especially for the treatment of tumor cells or other diseases in humans. In this

context, similar approaches have been reported recently. A nano-drug containing C-PC encapsulated in carboxymethyl-chitosan tagged to CD59sp was developed to treat the cervical cancer in *in vivo* female BALB/c nude mice and HeLa cells [123,165–166]. Peng and collaborators and Yan Wen and collaborators have encapsulated phycocyanin in an electrospun fiber, to prevent colon cancer. Furthermore, the addition of PC to well-established anti-cancer drugs could improve cancer therapy significantly [100,102].

#### 5.2.6. Biomedical use of PBPs

Inventions aimed at the application in biomedical sciences have been developed, due to their antioxidant and antitumor effects, among others. PBPs showed anti-oxidative properties both *in vitro* and *in vivo*. PC could effectively eliminate hydroxyl radicals ( $\bullet\text{OH}$ ) and alkoxy radicals ( $\text{RO}\bullet$ ) and inhibit lipid peroxidation [86,89]. Moreover, the selenium-containing PCs (Se-PCs) has shown an ability to scavenge superoxide and hydrogen peroxide radicals positively correlated with the Se content [167]. In this context, it has been developed a selenization process in *Spirulina*, useful for the treatments of inflammatory intestinal diseases [168]. As *in vivo* and *in vitro* PBPs have shown anti-tumoral properties, C-PC purified from *Oscillatoria tenuis* inhibited the growth of HT-29 (colon cancer) and A549 (lung cancer) cells, and induce apoptotic cell death [169]. Skin tumors can be induced by f12-O-tetradecanoyl-phorbol-13-acetate (TPA). In the early stage of tumor progression, oral administration of C-PC extracts from *Spirulina*, reverse the expression pattern of proteins related to cancer as interleukin-6 and pSTAT3 [170].

On the other hand, the administration of recombinant APC inhibited the size of tumors in H22 hepatoma model mice [171]. Similarly, recombinant PE could inhibit the growth of HeLa cells, concentration dependent [172]. These studies indicate that native PBP and recombinant PBP have potential medical value in anti-tumor applications. Additionally, oral formulations of PBPs polysaccharide extract have been developed for treatment or prevention of pancreatic cancer [173]. Also, PBPs are useful in photodynamic antitumoral therapies in skin cancer [174] also has been designed a methodology to obtain powered of PBPs in photodynamic antitumoral therapies, cosmetic and food industry [158]. Table 9 Summarizes the patents related to production, extraction, purification, and biomedical applications in different industries.

## 6. Future perspectives

Currently, there is great concern about the use of synthetic colorants in the food industry, due to the negative impact they can have on human health and the environment. In this regard, it has been demonstrated that Allura Red accelerates the formation of tumors in the immune system of mice and causes hyperactivity in children [189]. Indigo carmine has been associated with the development of brain tumors [190] and Sunset yellow may be related to hyperactivity and hypersensitivity in children [191]. In addition to their harmful effects on health, these synthetic colorants are difficult to remove from industrial wastewater, causing environmental damage. In this sense, the colorants obtained from natural sources are an attractive alternative. Thus, the natural colorant market is expected to reach 3.2 billion dollars by 2027. In this scenario, PBPs can provide coloring properties, along with diverse biological activities, providing added value to these compounds. However, the most common natural colorants used as food ingredients are obtained from plants or algae. This involves a large use of land or aquatic environments, long harvesting times and considerable energy consumption. As a result, a promising and economically viable alternative is to obtain these colorants from microbes. There are several successful examples of this strategy.

**Table 9**

Patents related to phycocyanins in the fields of production, extraction, purification, and biomedical applications.

Type of patent	Title	Reference
<b>Production</b>	Method for inducing the synthesis of PBPs.	[175]
	A recombinant photosynthetic protein molecule with a wide range of absorption and its construction process	[153]
	Recombinant phycobiliprotein fluorescent protein with large Stokes shift and preparation method thereof	[152]
<b>Extraction</b>	Methods for extracting and purifying <i>Nostoc sphaeroides</i> Kutzing phycobiliprotein, and purified phycoerythrin	[176]
	Extracting and purifying method for <i>Nostoc sphaeroides</i> Kutzing phycobiliprotein and purified phycoerythrin	[177]
	Extraction method of phycobiliprotein of <i>Nostoc sphaeroides</i> Kutzing	[172]
	Phycobiliprotein draws with many fallers corona discharge reaction unit	[179]
<b>Purification</b>	Technology for separating and purifying phycobiliprotein from <i>Gracilaria tenuistipitata</i> deep processing sodium solution	[180]
	Method for extracting and purifying phycobiliprotein and purified phycocyanin	[181]
	The process of achieving the highest degree of drug's purity of c-phycocyanin from <i>Spirulina</i> alga	[157]
	Kit for early detection of liver cancer and preparation method thereof	[182]
<b>Diagnostic application</b>	A kind of utilize recombinant detection reagent bar of fluorescence phycobiliprotein subunit and preparation method thereof	[183]
	Composition containing phycobiliprotein polysaccharide extract and use of a composition containing phycobiliprotein polysaccharide extract	[173]
<b>Pharmaceutical application</b>	Preparation method of phycobiliprotein polypeptide powder	[158]
	Antitumor drug carrier and application method thereof	[184]
<b>Clinical and cosmetic Application</b>	Preparation method of double-network hydrogel loaded with phycobiliprotein	[185]
	Anti-enteritis <i>Spirulina</i> selenylation	[168]
	Phycobiliprotein-polypeptide complex and preparation and application thereof	[186]
	Compositions for protecting skin comprising DNA repair enzymes and phycobiliprotein	[187]
	Heart valve prosthesis, preparation method, and <i>in-vivo</i> heart valve prosthesis sterilization method	[188]
	Phycocyanin composition for use in inhibiting bone resorption	[174]
	Novel material based on natural diatom shell and phycobiliprotein and application	[174]

Guerrero-Rubio and collaborators produced betalains (betacyanins (red-violet) and betaxanthins (yellow-orange)) from L-DOPA in a 2L bioreactor of *Escherichia coli* [192]. Wehrs and collaborators produced a natural blue pigment Indigoidine from *Saccharomyces cerevisiae* in a 2L bioreactor [193]. Another example was developed by Wang and collaborators, who engineered a tyrosinase from *Bacillus megaterium* into a bacterium *Vibrio natriegens*, resulting in a pigment similar to melanin [194]. In order to validate industrial production of these compounds and ensure maintenance of color, it is necessary to integrate omic techniques together with biological

synthesis and metabolic engineering. In addition to this, selecting microorganisms considered Generally Recognized As Safe (GRAS) is crucial to guarantee their safety. Finally, after taking into consideration the spectroscopic characteristics of PBPs discussed above, an interesting approach would be to use metabolic engineering to have a single microorganism producing PBPs of different colors, thus contributing to reduce the costs of obtaining natural colorants and enhance productivity.

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## CRedit authorship contribution statement

**Jorge Dagnino-Leone:** Conceptualization, Writing - original draft, Visualization, Writing - review & editing, Project administration. **Cristina Pinto Figueroa:** Writing - original draft. **Mónica Latorre Castañeda:** Writing - original draft. **Andrea Donoso Youlton:** Writing - review & editing. **Alejandro Vallejos-Almirall:** Writing - original draft. **Andrés Agurto-Muñoz:** Writing - review & editing. **Jessy Pavón Pérez:** Writing - review & editing. **Cristian Agurto-Muñoz:** Supervision, Funding acquisition, Resources.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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